We make it visible.

Epi-Fluorescence Illuminators IV FL & III RS

INSTRUCTIONS FOR EXCHANGE OF FILTERS

IV FL used with Standard microscopes: pages 1-4
III RS used with Universal Research & Photomicroscope: pages 5-6

Note:
Excitation and barrier (emission) filters have a diameter of 18mm with a max. thickness of 6mm.
Dichroic beam splitters have a diameter of 22mm with a max. thickness of 1mm.

Every Standard microscope can be converted into a fluorescence microscope for incident-light excitation with the epi-condenser IV FI (46 63 00-9901)[1]. Although primarily suited for transparent specimens on slides, it can be used to examine opaque specimens as well.

Depending on the application, you can choose from the following lamp types as exciting-light sources: HBO 50 W AC (38 16 19) or HBO 100 W/2 (38 00 18-4060) high-pressure mercury lamp, XBO 75 W/2 (38 00 79-9190) high-pressure xenon lamp, or 12 V 100 W halogen lamp (38 00 59-1660). Microscope illuminator 100 (see operating instructions G 41-310/III) is used as lamp housing of all above-mentioned lamp types.
A special feature of epi-fluorescence condenser IV FI is a 2-position sliding sleeve in the tube, which accepts reflector housing 2 FI with 2 complete filter sets each (see Table on p. 7).

With the reflector housing one changes quickly between two filter sets or between transmitted-light and fluorescence microscopy. The reflector housing can be easily exchanged for another one with different filter sets.

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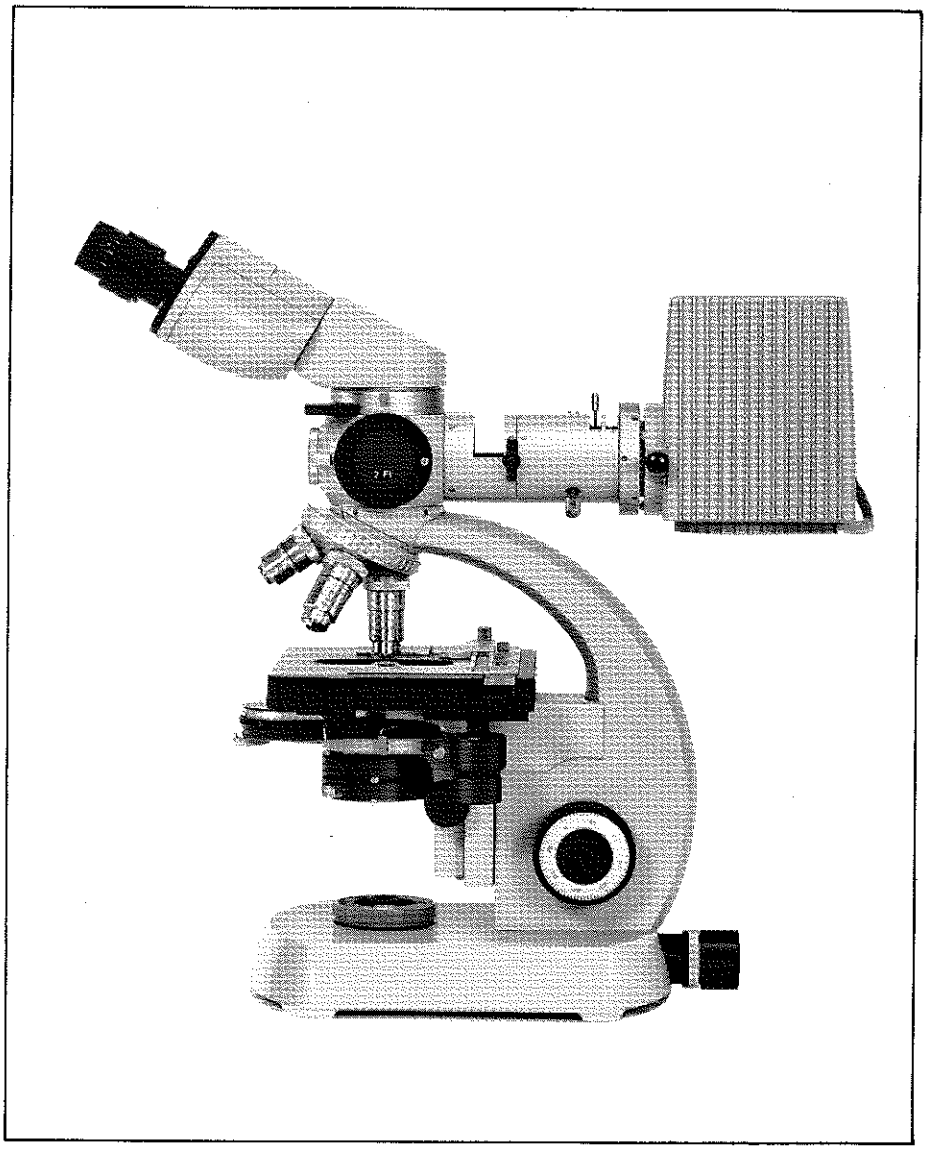


[1] The 6- or 10-digit numbers in brackets are ordering numbers which are imprinted on some modules.

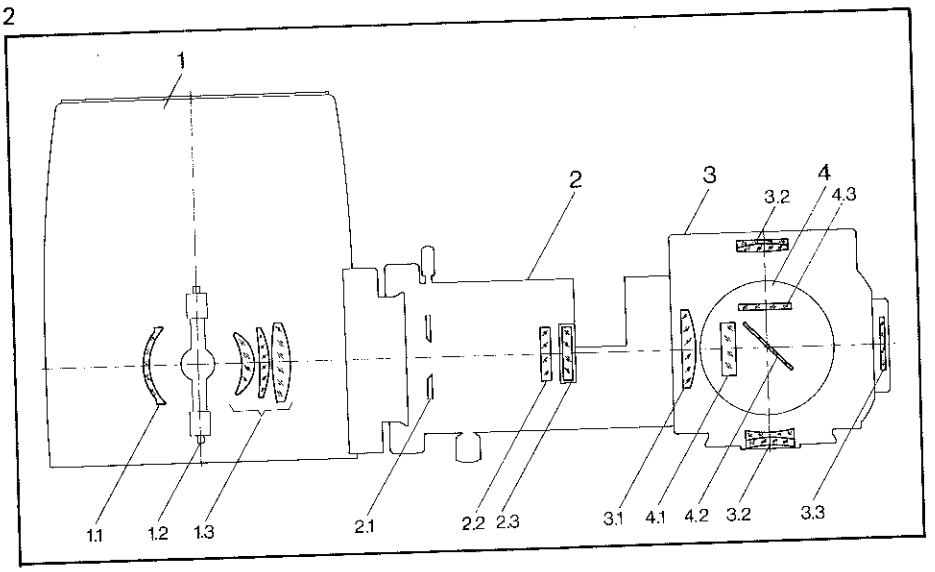
Fig. 2: Epi-fluorescence condenser IV FI, schematic

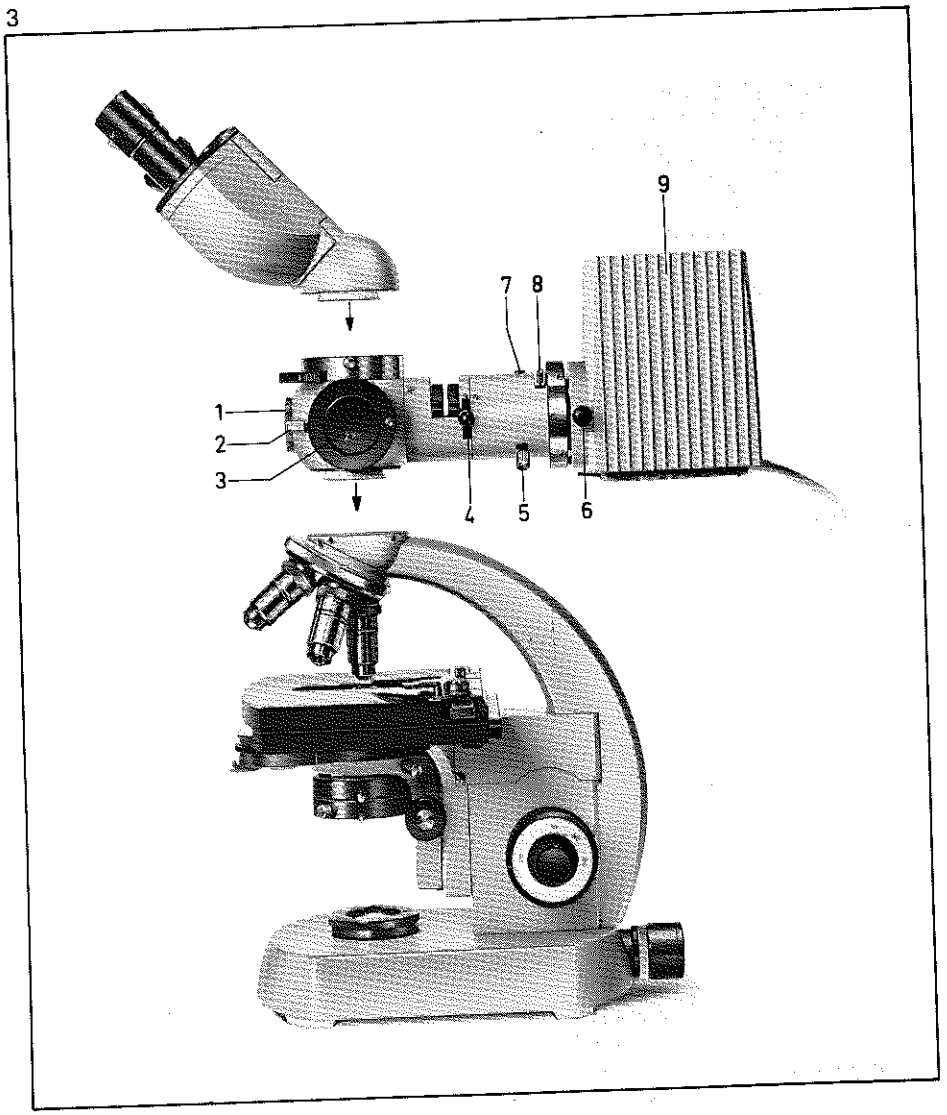
1 Illuminator 100
1.1 Concave mirror
1.2 Light source
1.3 Lamp condenser with several lens elements

2 Illuminating tube
2.1 Luminous field diaphragm
2.2 Heat-absorption filter
2.3 Three-position slider
position 1: no light passage
position 2: red-attenuating filter
position 3: free light passage
(filter holder)

3 Tube
3.1 Convergent lens
3.2 Telan lens system
3.3 Frosted, dark glass plate to check the lamp adjustment

4 Reflector housing 2 FI, combining in one unit:
4.1 exciter filter
4.2 reflector, a 45° plane glass acting as chromatic beam splitter
4.3 barrier filter





Assembly

Before mounting the epi-fluorescence condenser IV FI on the microscope, fit illuminator 100 (3.9) [1] to dovetail ring of illuminating tube and secure with clamping screw.
Insert the entire unit in the tube port of the microscope, and mount the viewing tube on top. Secure all dovetail connections with knurled screws.
Slide reflector housing 2 FI (3.3) into sliding sleeve of tube as far as it will go and secure with screw (3.2).

[1] (3.9) means Fig. 3, item 9

Adjusting illumination and fluorescence image

Adjusting the illumination with HBO 50 W high-pressure mercury lamp
The filter set for green or light-blue excitation is the best choice for adjustment.

Connect lamp to its power supply and switch ON. The HBO 50 ignites automatically, and is ready for adjustment after 2 to 3 minutes warm-up time.

Set slider (3.4) to position 3 (free light passage). Focus on the feature on groundglass (3.1) by focusing the lamp condenser with knob (3.6). The sharpness of the light-source images can be improved by closing the luminous field diaphragm (3.8).
Center direct and reflected light-source images next to one another on groundglass (3.1) by moving the lamp socket vertically and laterally, and by focusing and tilting the concave mirror (Fig. 4) (see also operating instructions G 41-310/III).

The centering of the illumination can be controlled on the groundglass but need hardly ever be corrected. For fluorescence observation change the focusing of the lamp condenser so that the image of the object field is uniformly illuminated.

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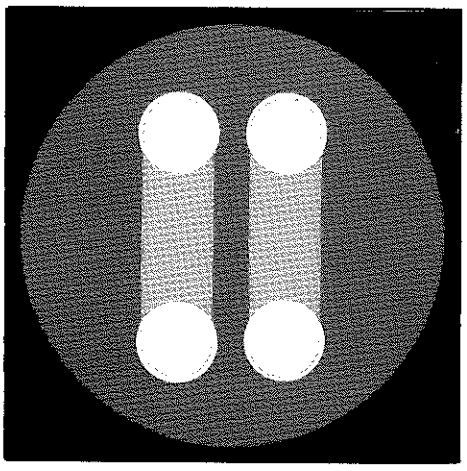

Fig. 4: Centered focal spots of HBO 50 W high-pressure mercury lamp and their mirror image

Adjusting the fluorescence image

Fluorescence observation will be easier when the following hints are observed:
Start with low-power objective, preferably Neofluar 10/0.30. Use a strongly fluorescent object. Use as reference for the adjustment at first the paper label which sticks on many specimens.
Such specimens help considerably when checking the uniformity of the illumination.

Close luminous field diaphragm with lever (3.8). Focusing of the diaphragm is possible after loosening slot screw (3.7). Center the diaphragm in the field with centering screws (3.5) on both sides. Open and close luminous field diaphragm as necessary; because of the parfocalization of the objectives, the focus of the luminous field diaphragm is maintained when objectives are exchanged.

Recommended optical equipment 5

In the epi-fluorescence condenser IV FI the fluorescence-exciting radiation is directed on to the specimen from above through the objective which is used for observation as well. Incident-light bright-field excitation is thus realized. With this method, the following factors must be considered in practice:

1. The objectives must let pass through sufficient exciting radiation and must have little intrinsic fluorescence.

2. The illuminating aperture is equal to the observation aperture.

Achromat and Neofluar objectives fulfil the requirements under item 1. above, whereas Planachromat and Planapochromat objectives with their higher degree of correction are not as universally applicable as the first-mentioned objective types.
It follows from item 2. above that low-power, low-aperture objectives are less suited. Always use the objective with higher aperture if you have the choice of objectives of equal magnification but different aperture, i.e. use a Neofluar 10/0.30 instead of an Achromat 10/0.22 objective, or a Neofluar 40/0.75 instead of an Achromat 40/0.65 objective.

The best choice are Plan-Neofluar objectives which have been specially developed for fluorescence microscopy. We recommend to use a combination of a high-power objective and a low-power eyepiece, because the brightness also depends on the microscope magnification. But, as with other methods in microscopy, the best observation conditions are achieved with medium-power eyepieces in the range between 8x and 12.5x.

Epi-fluorescence microscopy is possible in conjunction with transmitted-light techniques such as phase contrast, dark-field, differential-interference contrast and polarization. Any transmitted-light condenser can be used for the purpose.

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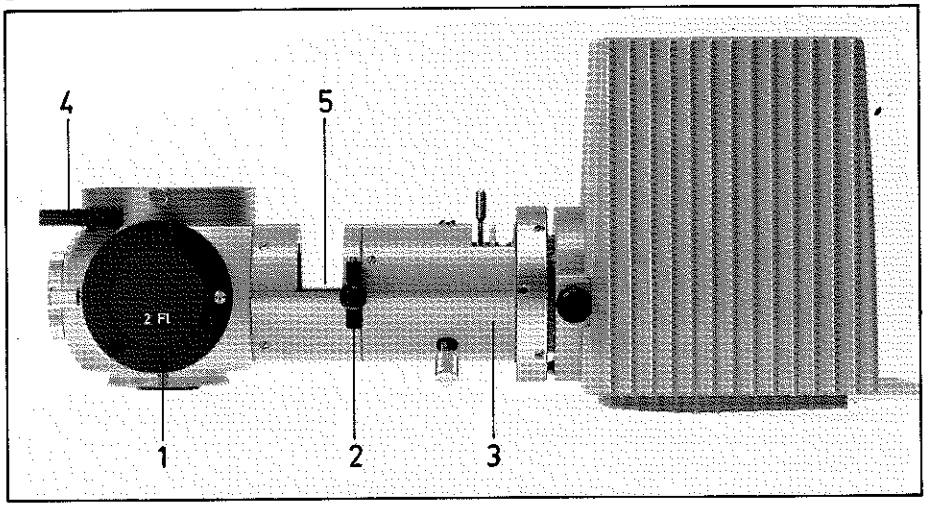

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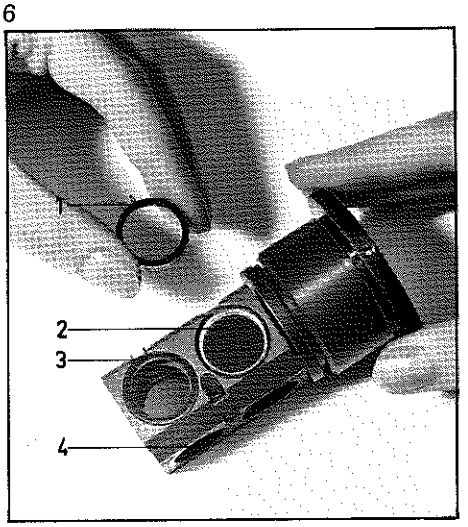


Fig. 6: Filter exchange of double reflector housing; cover plate removed

A solid-glass filter KG 1 is provided as heat-absorption filter (**2.2**) in the light path to protect the other filters and the specimen. It is easily exchanged by removing the tube part (**5.3**) carrying the luminous field diaphragm.

The shutter and filter slider (**5.2**) accepts two 18 mm dia. filters. The basic equipment includes a red-attenuating filter BG 38 in one opening of the slider, whereas an 18 mm dia. filter, max. 5 mm thick fits into the other opening.

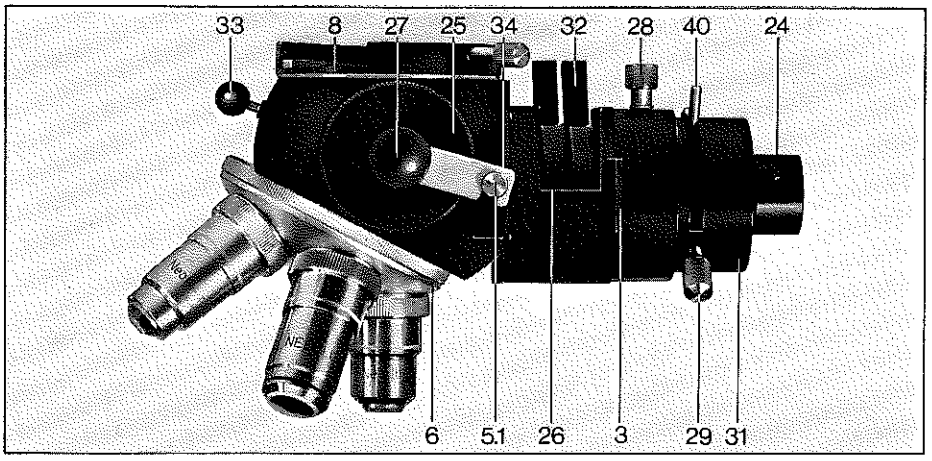
Additional exciter filters can be loosely fitted in filter holders (**5.5**). Use ring (46 72 52) for 32 mm dia. filters, and, in addition, adapter ring (46 78 93) for 18 mm dia. filters. Max. filter thickness for each ring 5 mm.

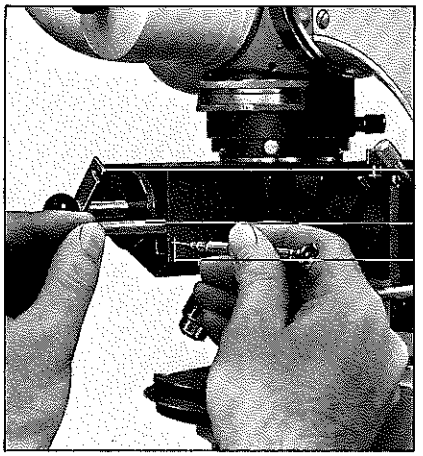
The most frequently used exciter filters, chromatic beam splitters and barrier filters are exchangeably mounted in reflector housing 2 Fl (**5.1**). All exciter and barrier filters listed on p. 7 are 18 mm in diameter. They are kept in place by plastic rings with dogs. The filter holders accept max. 6 mm thick exciter filters (**6.2**) and max. 4 mm thick barrier filters. Thinner filters are supplied with spacer rings.

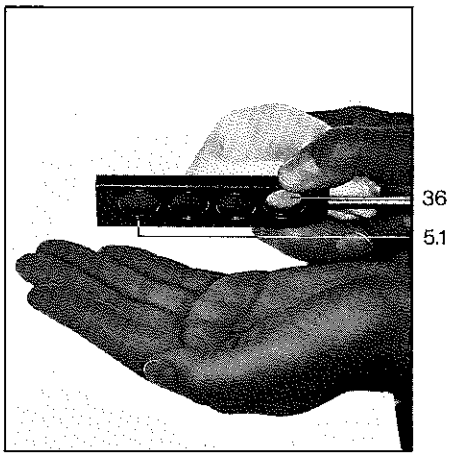
To exchange a filter, disengage both dogs (**6.3**) from their notches and pull out plastic ring (**6.1**). The loose filters and spacer rings, if any, can be easily exchanged.
Insert the ring of max. 2 mm thick filters with the seating edge on the inside, and that of thicker filter rings on the outside. If a filter has play, place a spacer ring between filter and ring.

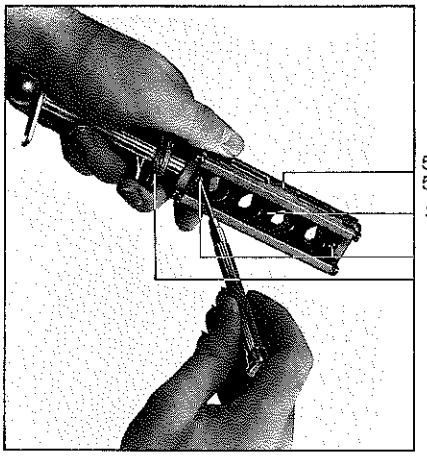
The 45° interference filters which act as chromatic beam splitters, have diameters of 22 mm. They are loosely fitted in their holders and accessible after loosening two screws. The highly sensitive interference layers being unprotected on the top surface of the beam splitters, these should be left in place.
Shutter and filter slider (**5.4**) 20x6 mm, above the barrier filters accepts additional 18 mm dia. barrier filters max. 5 mm thick.

Filters and beam splitters









The heat-absorbing filter (2) (see diagram on page 5) is a KG 1 solid glass filter. It should remain constantly in the light path for protection of the other filters and the specimens, so there is no facility for removing it. It is easily exchanged, if necessary, however, by removing the lamp field stop insert.

The light-tight and filter slide (3) has two free passages for filters of 18 mm dia. The BG 38 red-attenuation filter is supplied with the basic equipment. The second opening can take any filters of 18 mm dia. up to a thickness of 5 mm, such as the KP 600 or KP 560 minus-red interference filters. These eliminate the red more stringently than the BG 38 attenuation filter, but have reduced UV transmittance.

Additional exciter filters can be inserted in the filter slots (26). For filters with 32 mm dia. the holding ring (46 72 52) should be used; for those with 18 mm dia. the adapter for 18-to-32 mm dia. (46 78 93) as well. Maximum filter thickness for either holding ring is 5 mm.

Exciter filters, beam splitters and barrier filters are mounted in a slide which contains 4 filter sets, 2 of which can be rapidly exchanged. Pull knob (33) to bring in the other two filter sets alternately. When the individual filter sets are to be brought into the beam path successively, pull out knob (33) and turn it to the left; this overruns the stop position. In the basic equipment, the arrangement of the filters is such that with increasing wavelength the different types of excitation follow each other (see table on page 12).

All 18 mm dia. exciter and barrier filters listed in the table are easily exchanged. Unlatch lever (34) and loosen screw (35); the exciter filter slide (5.1) can be pulled out.

With a linnen cloth push the filter through the wider opening (36) and insert the new one. Exciter filters may be up to 6 mm thick. When using exciter filter sets, insert the interference filter first (coated surface towards the light source), then color glass or sandwich filters. Then insert slide (5.1); make sure that the filter flanges point towards the light source.

Unscrew cover (25) to pull the reflector-and-barrier-filter slide (5.2, 5.3) from the tube of the condenser III RS. After loosening screws (3), remove cover (38), and exchange the barrier filters. The barrier filters may be up to 2.2 mm thick.

The reflectors are multi-layer interference filters with steep edges, which are rigidly mounted and adjusted in our factory. We recommend to keep these beam splitters in their positions. Reflectors should be exchanged in our factory or in the workshops of our representatives only.